**Serial No. 09/980,193**
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## REMARKS

Applicants' invention is a reaction medium for a fermentation process which utilizes a phase inversion temperature microemulsion as at least one of a carbon source or a substrate. The use of a microemulsion as a carbon source or a substrate provides many advantages to the fermentation medium for the fermentation process.

As is well known in the art, microemulsions have extremely low viscositles. The low viscosity of the microemulsion provides a reaction medium or a fermentation process with a lower viscosity which improves the incorporation of air into the process with less energy. In addition, due to the small particle size of the oil droplets in the microemulsion, the interfacial area between the oil phase and water phase is substantially increased which provides for better oxygen transfer and access of the micro-organisms to the oil component.

The prior art references cited by the Examiner neither teach nor suggest utilization of a microemulsion of a fatty acid alkyl ester or a vegetable triglyceride as a carbon source or a substrate in a fermentation process.

It is well known and understood in the art that carbon sources or substrates for transformation are generally present in a reaction medium for a fermentation process in substantial amounts since the carbon source or the substrate are utilized in the process to provide energy to the micro-organisms or to be the basis for a modified substrate. The carbon sources and the substrate are generally present in fermentation medium in the range of from about 2% to about 5 or 6% by weight of the fermentation medium. That is,

the components are present in a concentration of from about 20 grams per liter to about 60 grams per liter of the reaction medium. Applicants respectfully submit that the prior art references cited by the Examiner neither teach nor suggest including in a fermentation medium a carbon source or a substrate which is either a fatty acid alkyl ester or a vegetable triglyceride as a microemulsion in the range of 20 to about 60 grams per liter of the alkyl ester or vegetable triglyceride in the fermentation medium.

Claims 12-35 are presently in the application. Claims 12-22 are considered on the merits. Claims 22-35 have been withdrawn from consideration as directed to a non-elected invention. Since claims 12-22 are allowable, Applicants request that the Examiner reconsider the requirement for restriction.

Claims 12-22 are directed to a reaction medium for fermentation process which comprises:

(a) a micro-organism;

(b) a phase inversion temperature emulsion, wherein the emulsion comprises water, an emulsifier and an oil phase selected from a group consisting of (i) fatty alkyl esters, vegetable triglycerides and mixtures thereof comprising at least one of a carbon source or a substrate and, wherein, the emulsion has an average droplet size of from 50 to 400 nm (microemulsion).

Applicants respectfully submit that the prior art references cited by the Examiner neither teach nor suggest the present invention.

Claims 12-22 stand rejected under 35 USC 103(a) as unpatentable over Inlow et al.

(US 5,372,943) taken with Kopp-Holtwiesche (DE 3738812) and Forster et al. (WO 95/11660). Applicants respectfully submit that Inlow et al., Kopp-Holtwiesche and Forster et al. whether considered alone or in combination neither teach nor suggest the present invention.

The Examiner states:

> "Inlow et al. discloses a reaction medium comprising a microemulsion and the microorganisms insect cells wherein the size distribution overlaps and which contains the same ingredients (see e.g., Example 8, Example 9 and Example 2). The reference differs from the claimed invention a fatty acid methyl ester is not used in the medium. However, Kopp-Holtwiesche discloses a similar microbial reaction medium containing methyl laurate or other fatty acid methyl esters (See e.g., Examples 1 and 3)."

Applicants respectfully request that the Examiner reconsider her understanding of the teachings of Inlow et al.

As is known in the prior art and as shown in the references cited in the present application, carbon sources and substrates are present in fermentation medium in a range of from about 20 to at least about 60 grams per liter of the fermentation medium.

In contrast to the teachings of the prior art concerning the amounts of the carbon sources and substrates required in the fermentation medium, Inlow et al. teach that the lipids are required in extremely small quantities in the fermentation medium. Applicants invite the Examiner's attention to column 12, lines 41-48 wherein Inlow et al. teach:

> "The reason lipids are supplied to the cells is not critical. They can be supplied as a microemulsion according to this invention where they are considered as essential or growth promoting nutrients, physical or chemical protectants or as having other functions, as for example, as a solvolytic agent, as a membrane modifying agent, as a surface tension reducing agent and/or as a cell surface stabilizing agent."

Because of the function of the lipids utilized in the Inlow et al. process, the lipids are present in a range of about 1-50 mg per liter and preferably 5-15 mg per liter (col. 5, lines 10-15) of the fermentation medium. This is in contrast to the present invention which utilizes the fatty acid esters or triglycerides as a carbon source or a substrate which are generally present in fermentation medium in a range of from about 20 to about 60 grams/liter. Applicants therefore respectfully submit that the microemulsion of Inlow et al. cannot be considered as a carbon source or a substrate in the fermentation process.

It is clear from the specification and in Figure 1 that the microemulsion of Inlow et al. increases the growth rate of the cells in the culture. Even though the fatty acid or triglyceride portion of the microemulsion is present in the fermentation medium in a concentration of in the range of about 1 mg per liter, the fatty acid or the triglyceride remains in the fermentation medium for at least about 160 hours. Applicants submit that the presence of the small amount of the fatty acid or the triglyceride in the fermentation medium for such a long period of time would indicate to one skilled in the art that the fatty acid or the triglyceride was not a carbon source or a substrate since it would be rapidly utilized as the carbon source or been transformed by the fermentation. Applicants respectfully submit that there is neither teaching nor suggestion in Inlow et al. that there would be any advantage for utilizing a microemulsion of a fatty acid ester or a triglyceride as a carbon source or a substrate in a fermentation medium. Applicants submit that the only connection between the teachings of Inlow et al. and the present invention is the addition of a microemulsion of a fatty acid or a triglyceride to a fermentation medium. The

purposes for which the microemulsion is introduced into the fermentation medium are different and the amounts of the fatty acid or the triglycerides introduced into the fermentation medium by way of the microemulsion are substantially different. At a minimum, the present invention requires that the fatty acid ester or the triglyceride be introduced into the fermentation medium in a concentration of several thousand times greater than the amount introduced into the Inlow et al. process. Applicants respectfully submit that Inlow et al. would neither teach nor suggest utilizing a microemulsion of a fatty acid ester or a triglyceride as a carbon source or a substrate in a fermentation medium.

Applicants submit that as shown in the examples in Inlow et al., the presence of one milligram of a fatty acid or a triglyceride per liter of the fermentation medium over a period of at least 160 hours, would neither teach nor suggest that the fatty acid or triglyceride was a carbon source or a substrate. In fact, Applicants submit that the fatty acid or the triglyceride could not be a carbon source or a substrate since the presence of one milligram of the lipid per liter of the fermentation medium over at least 160 hours would indicate that the fatty acid or the triglyceride could not be a carbon source or a substrate since it would have been used as the carbon source or transformed rapidly in the fermentation process. Applicants further submit that there is neither teaching nor suggestion in Inlow et al. of any advantage for introducing a microemulsion of a carbon source or a substrate into a fermentation medium.

The deficiencies of Inlow et al. are not cured by combination with Kopp-Holtwiesche. Kopp-Holtwiesche discloses the use of methyl laurate as a substrate for forming alpha-

omega dicarboxylic acids by a fermentation process. However, Kopp-Holtwiesche neither teaches nor suggests introducing the methyl laurate into the fermentation process as a microemulsion.

As disclosed in Kopp-Holtwiesche, a fermentation medium is formed containing in a range of from about 2 to 3% glucose as a carbon source and minor nutrient materials including an emulsifier to form the fermentation medium. The *candida tropicalis* microbes are introduced into the fermentation medium and permitted to propagate until the glucose has been substantially reacted by the microbes. Then a mixture of a yeast-nitrogen base, an emulsifier, methyl laurate and a phosphate buffer are introduced into the fermentation medium as a substrate which is transformed by the *candida tropicalis* microbes into the alpha-omega-dicarboxylic acid ester.

There is neither teaching nor suggestion that the methyl laurate be introduced into the fermentation process as a micro emulsion nor any advantage for making such an addition. Therefore, Applicants submit that Kopp-Holtwiesche does not cure the deficiencies in Inlow et al. and neither teaches nor suggests the present invention.

The deficiencies in the teachings of Inlow et al. and Kopp-Holtwiesche are not cured by further combination with Forster et al. Forster et al. is directed to finely dispersed oil-in-water emulsion containing an organic cosmetic active agent by forming an emulsion of (A) an oil body with (B) 0.5 to 30 wt % of a non-ionic emulsifier with a hydrophile-lipophile balance of 10 to 18 and (C) or 0.1 to 30 wt % of a co-emulsifier from a group of fatty alcohols with 12 to 22 carbon atoms or partial esters of polyols with 3 to 6 carbon atoms

with fatty acids with 12 to 22 atoms and (D) an organic cosmetic active agent selected from a group of deodorizing agents, perfume oils and light-protective factors, provided that i) the sum of components (A) and (D) amounts to 10 to 90 wt % and ii) the proportion of the component (D) amounts to 5 to 100 wt % in relation to the oil body (A), is emulsified in the presence of 8 to 85 wt % of water at a temperature above the melting point of the mixture of components (A) to (D) and the emulsion is heated to a temperature within or above the phase inversion temperature range, or the emulsion is produced at this temperature, after which it is cooled to a temperature below said phase inversion temperature range and possibly further diluted with water.

Applicants respectfully submit that Forster et al. does not cure the deficiencies in the combination of Inlow et al. and Kopp-Holtwiesche. In the official action, the Examiner characterizes Forster et al. as the preparation of a fermentation medium. However, Applicants respectfully submit that the Examiner is stretching the concept of a fermentation medium as required in the present application. Forster et al. generally would teach one skilled in the art that a finely dispersed oil-in-water emulsion could be prepared by PIT method. However, one skilled in the art would realize due to the presence of the perfumes, deodorizing agents and light-protective factors in the emulsion, that it would not be suitable as a fermentation medium useful in the practice of the present invention.

Applicants submit that one skilled in the art would realize that the extraneous ingredients which are required in the Forster et al. composition, could have a deleterious effect on the microbes in a fermentation process. In fact, the emulsions containing organic

cosmetic active agents, generally contain anti-microbial materials to prevent fermentation of the composition. Applicants submit that one skilled in the art would not attempt to provide a useful fermentation process based on the oil-in-water emulsion disclosed in Forster et al. with its cosmetic active ingredients.

According to the Examiner's speculation, every organic substance associated with water would constitute a fermentation medium for a fermentation process. Literally, in most of the situations, although fermentation can occur, the fermentation is unintended, unwanted and generally is deleterious to the composition. Applicants therefore respectfully submit that a combination of Forster et al. with Inlow et al. and Kopp-Holtwiesche is untenable.

To form a rejection over a combination of references, there must be some teaching or suggestion to make the combination. Applicants respectfully submit that Forster et al. would teach one skilled in the art away from the present invention since fermentation of the composition would be unintended, unwelcome and would spoil the composition. Applicants therefore respectfully request that the rejection be reconsidered and withdrawn.

In forming the rejection, Applicants submit that the Examiner has completely failed to consider the nature of the various compositions in the references considered. Inlow et al. discloses introduction of a small amount (1 mg per liter of fermentation medium) of a fatty acid or a triglyceride as a growth promoter for the microbes in the fermentation medium. The small amount of the fatty acid or the triglyceride in the fermentation medium and the fact that it remains in the fermentation medium for an extended period (at least 160

hours) would teach one skilled in the art that the fatty acid or the triglyceride was not a carbon source or a substrate. Therefore, there would be no teaching or suggestion to introduce a microemulsion of a fatty acid ester or a triglyceride in major amounts into a fermentation medium for the purpose of a carbon source or a substrate.

The deficiencies in Inlow et al. are not cured by Kopp-Holtwiesche. Kopp-Holtwiesche merely discloses introducing a fatty acid ester into a fermentation medium which contains the nutrients and an emulsifier. The fatty acid is introduced into the fermentation medium in a mixture with a nutrient material, an emulsifier and a phosphate buffer. However, there is neither teaching nor suggestion that the single emulsifier would teach one skilled in the art that the fatty acid ester (methyl laurate) was not introduced into the fermentation medium as a microemulsion. Applicants therefore respectfully submit there is neither teaching nor suggestion in the combination with Inlow et al. and Kopp-Holtwiesche that the carbon source or the substrate be introduced into the fermentation medium in the form of a microemulsion.

Forster et al. is not pertinent to the present invention and would neither teach nor suggest to make the combination of references since it is merely directed to a finely dispersed oil-in-water emulsion which can be prepared by PIT method. There is neither teaching nor suggestion that it is a fermentation medium or that there would be any advantage of introducing it into a fermentation medium. As disclosed, one skilled in the art would understand that one preparing the Forster et al. composition would take precautions to prevent micro-organisms from entering the composition.

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Applicants respectfully submit that the Examiner's rejection is based on a misreading or misunderstanding of the claims. At the fourth paragraph of page 4 of the official action, the Examiner states:

> "Regarding the argument that the microemulsion could not be useful as a carbon source or as a substrate, it is noted with all due respect that the claims now specifically comprise at least one of a carbon source or substrate, which is independent of the microemulsion. Therefore this argument is without merit."

Applicants respectfully request that the Examiner reconsider claim 12. Claim 12 (b) reads as follows:

> "A phase inversion temperature emulsion, wherein the emulsion comprises water, an emulsifier and an oil phase selected from the group consisting of (i) fatty acid alkyl esters, vegetable triglycerides, and mixtures thereof comprising at least one of a carbon source or a substrate, and wherein the emulsion has an average droplet size of from 50 to 400 nm."

If the phrase is read in its context, the phrase comprising at least one of a carbon source or a substrate clearly refers to the fatty acid alkyl esters, vegetable triglycerides and mixtures thereof which is the oil phase of the phase inversion temperature emulsion. The phrase "comprising at least one of a carbon source or a substrate" cannot be read in a vacuum but must be read in the context of the claim as written. Applicants therefore respectfully submit that the carbon source or substrate is the oil phase of the phase inversion temperature emulsion. Applicants respectfully request that the Examiner reconsider her understanding of the claims in the present application.

In the fifth paragraph at page 4, the Examiner attempts to justify the rationale that Forster et al. discloses a reaction medium for a fermentation process. Applicants submit

that Forster et al. would teach one skilled in the art away from utilizing the dispersion as a reaction medium for a fermentation process. Applicants would appreciate the Examiner pointing out some reference in the prior art which teaches that the cosmetic compositions of Forster et al. have any utility in the real world as reaction medium for a fermentation process. Under the Examiner's analysis, any organic compound which is associated with water is a reaction medium for a fermentation process. However, the Examiner fails to consider that in the "real world" a cosmetic formulation such as disclosed in Forster et al. contains anti-microbial agents to suppress or eliminate fermentation in the medium. Applicants therefore respectfully submit that the Examiner is classifying any organic material in contact with water as a reaction medium for a fermentation process. Applicants respectfully submit that this is not in conformation with what one skilled in the art would understand.

At the penultimate paragraph on page 5 of the official action, the Examiner states:

> "With respect to applicant's arguments that one of ordinary skill in the art would not have modified the culture medium of Inlow according to the teachings of Kopp-Holtwiesche because lauric acid methyl esters are not part of an emulsion used in the reaction medium. Inasmuch as the amount of emulsion contained in the present culture medium is not disclosed, it is uncertain that all of the medium is emulsified. It is noted that the compositions of Kopp-Holtwiesche would have been reasonably expected by one of ordinary skill in the art to be naturally emulsified at least to some extent as a consequence of agitation (See e.g., Examples). In addition a reaction medium is no more than a composition that supports the growth of a microorganism."

Applicants respectfully request that the Examiner reconsider her interpretation of the combination of Inlow et al. with Kopp-Holtwiesche in connection with the present invention.

As presently claimed, the present invention is limited to use of a phase inversion temperature (PIT) emulsion having an average droplet size of from 50 to 400 nm. From reading the examples in Kopp-Holtwiesche, Applicants are not certain that the ethyl laurate is introduced into the fermentation medium in the form of an emulsion. However, if the methyl laurate is introduced into the fermentation medium in the form of an emulsion, it is certainly not an emulsion with a particle size in the range of 50 to 400 nm. One skilled in the art reviewing the examples in Kopp-Holtwiesche, would realize that the amount of emulsifier used in the fermentation medium is relatively small (0.2%). The small amount of the emulsifier in the fermentation medium would clearly teach one skilled in the art that the carbon source or the substrate was not introduced into the fermentation medium in a form of emulsion having a droplet size of from about 50 to 400 nm. Applicants therefore respectfully request that the Examiner reconsider the teachings of a combination of Inlow et al. with Kopp-Holtwiesche.

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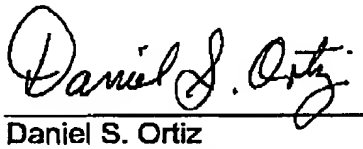
In view of the above discussion, Applicants respectfully submit that a rejection of the claims over Inlow et al. in combination with Kopp-Holtwiesche and Forster et al. is untenable and respectfully request that the rejection be reconsidered and withdrawn.

Respectfully submitted,



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